CHAPTER I
INTRODUCTION
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Genesis of the present project. A number of nutritional deficiency diseases are known to be commonly prevalent in the industrially developing countries of the world. In comparison, several diseases of plenty have also arisen in the industrially developed affluent communities of the West. An excessive amount of body cholesterol has been recognized as one such factor. Excessive body cholesterol has in recent years been implicated in a number of serious and dreaded biochemical disorders of the cardiovascular and the central nervous systems, in gallstone formation, and also in the etiology of certain forms of cancer (1). Recent researches have identified this excessive cholesterol to be mostly of exogenous influx, from the cholesterol rich diet, and arising, to a fair degree, from a deficiency of vitamin C (L-ascorbic acid, AA); the latter might not be apparent to the extent of its severe form usually known as scurvy, but might be present to the extent of only a lower order, which at present is referred to as latent marginal AA deficiency. In fact, hypercholesterolemia, atherosclerosis, and even coronary heart disease have currently been claimed to be vitamin C deficiency diseases (2). The recently developed hypotheses, under the terms molecular biology (3) and radical biochemistry/pathology (4) of AA-cholesterol relationship tend to support the view point that mega doses of AA could prevent/suppress the above
disorders of excessive cholesterol, and many other allied defects. Szent Gyorgyi-Stone-Pauling school advocates daily multi-gram doses of AA for modern man for full expression of his maximum potential for health, and for ability to resist the present day stresses - the biochemical stresses so common in our modern society-, and for bodily malfunctions (3). In this connection, it may be pointed out that the situation in the oriental societies, hitherto assumed as safe, might not in reality be very far from this, and these aspects might not be limited anymore only to the West. This situation was the genesis of the present study.

**Rationale and scope of the proposed investigation.** Every cell of the human system contains cholesterol, as it is an essential requisite for the normal structural integrity and the physiological functions of the cellular membranes; every cell is also capable of synthesizing it with the possible exception of matured mammalian erythrocyte, as well as exercising a control over the activities of some 26 enzymes responsible to elaborate it from active acetate. The average daily metabolic requirement of cholesterol of an adult is known to be around 350 mg; as against this, the daily consumption from the animal-based diet (like that of the Westernman) is around 500 mg plus an assorted amount of other sterols ("phytosterols"); this input has to be considered along with an average daily synthesis of cholesterol (endogenous cholesterol, c. 1.0 gm). The total daily influx thus comes to c. 1.5 gm cholesterol. This excess cholesterol accumulates in his average 70 years of life, and ultimately
turns out to be a slow and silent killer, mostly affecting his cardiovascular system. Incidentally, it might not be out of place to point out that although cholesterol had hitherto been considered as exclusively an animal sterol, since 1963 it has been recognized to be a typical plant sterol as well. Of course, it is true that up to now it has been found to occur in a few plant species only, but it is not impossible that it could be present in many others too; and these phytosterols, as well as the cholesterol precursors, also might prove as harmful as cholesterol itself. It is heartening to find that phytosterols not only remain unabsorbed but also hinder the absorption of dietary and or exogenous cholesterol.

The amount of cholesterol (mg/100 gm) present in the common animal vs. vegetable foods seems to support the above postulate:

- brains 2000, meats 65-75 (beef 75, lamb 72, pork 65),
- egg yolk 1500, butter 250, milk 14, cheese 7-113.
- yoghurt 8. In contrast, cereals, pulses, legumes, vegetable oils, vegetables, fruits, etc. 1-4, mostly as phytosterols.

An average adult human body (70 kg) contains c. 145 gm cholesterol, i.e. about 0.2% of his body weight. Of this amount, 8 gm (5.5%) is contained in his blood plasma, as lipoproteins. The total cholesterol content (per cent) of the major classes of human plasma lipoproteins has been found to be: chylomicrons 10.5, VLDL 17, LDL 41 and HDL 11(1).

It thus becomes clear that the LDL fraction is the main
cholesterol carrying component of plasma. Considerable evidence has now been accumulated which suggests that the incidence of arteriosclerotic coronary heart disease is positively related to plasma concentration of total cholesterol and LDL-cholesterol, but inversely related to HDL-cholesterol (5). Likewise, hyperlipidemia is known to play an important role in the induction and development of atherosclerosis (6-9), and therefore the serum lipid level must be normalized to prevent or treat atherosclerosis. The role of different lipoprotein fractions in the pathogenesis of atherosclerosis has also been elucidated: VLDL and LDL fractions are considered to be atherogenic, while the HDL fraction is thought to be anti-atherogenic (10-12). In patients with coronary heart disease, LDL-cholesterol has been reported to be significantly higher, and HDL-cholesterol significantly lower than found in the control groups (13).

Thus, the information and the reports detailed above establish and affirm the rationale and the scope of the proposed study.

**Scheme of studies:** In view of the insight gained from the information detailed above, it was planned

1) to undertake an experimental study on AA-cholesterol relationship, so designed as to limit itself to the isolated and specific central problem of investigating the effect of ingestion of AA on
blood cholesterol in hypercholesterolemic rats, both in the absence as well as in the presence of a potent hypocholesterolemic agent, focusing attention on whole blood and plasma on the one hand, and on erythrocytes and erythrocyte membrane on the other. This was expected to afford a reasonably complete picture of the possible role, if any, of AA in such a metabolic state.

The erythrocyte membrane was included in these investigations, because this membrane and the lysosomal membrane are thought to have common properties (14). Stabilization of the cell membrane is considered to be connected with a decrease in the inflammatory reactions, and the stabilizing effect of the acidic non-steroidal anti-inflammatory drugs on erythrocyte membrane has been shown to be due to their stabilizing effect on certain proteins in the membrane. The inflammatory response has been thought of as the first response of the cell to damage, and in the present case both AA as well as the hypocholesterolemic agent used (gum guggal) happened to be acidic non-steroidal anti-inflammatory agents. These aspects were thus expected to interconnect and link
some of the observations.

ii) to investigate the above mentioned effect of AA in a rather unconventional (or at least not so conventional) experimental hypercholesterolemia, induced by feeding cholesterol dissolved in propylene glycol (propane-1,2-diol, PG) as a solvent in place of the traditional oils/fats used for this purpose. This would cover some newer ground with respect to lipids vs. non-lipids as solvents (or cosolvent or additives) used in conjunction with the feeding of cholesterol.

Oils/fats (pure lipids) are known to exercise their own sharp and significant effect, in that they increase the intestinal absorption of cholesterol, and thence its levels in the blood, thus vitiating the final results obtained by their use. In contrast, PG, being a composite lipophilichydrophilic non-lipid solvent/additive, could possibly give different results. It may, however, be pointed out that, although PG had hitherto been considered inert and safe, it has of late been shown to be fairly active (or at least no so inert or inactive) as regards its own effect on the blood vs. tissue cholesterol relationship (15). Nevertheless, irrespective of these newer findings, it could certainly be
argued that even the composite data afforded by PG could still constitute newer contribution to the existing knowledge in this area, and thus might prove useful in providing some newer understanding of the central problem of AA-cholesterol relationship; and

iii) to examine the effect of a well known and proven ancient Indian indigenous drug gum guggul as the hypocholesterolemic agent, in place of the usual synthetic ones in current use; this would afford additional newer data.

Gum guggul had been used since long in the indigenous systems of medicine in the treatment of rheumatism; recent systematic scientific investigations have substantiated and confirmed its strong anti-inflammatory, hypocholesterolemic and hypolipidemic, anti-arthritic, anti-atherosclerotic, anti-myocardial infarction and anti-obesic activities; it is virtually nontoxic, and unlike the current synthetic drugs like clofibrate, is free from side effects. It has proved its worth in clinical studies as well (16,17); it compares fairly well with clofibrate. As the mode of action of gum guggul as a hypocholesterolemic agent could possibly be quite different from the actions of those in current
clinical use, its inclusion in our studies on AA-cholesterol relationship was likely to afford some newer insights in this field.

**Plan for its execution**: In order to execute the above scheme of studies, it was further planned that, as the first step, the lacunae in our current knowledge about these aspects be pinpointed from a critical analysis of a fairly comprehensive review of the existing literature on the subject used as a guide to the evaluation of the present status of the problem; this could, in turn, delineate the specific objectives to be achieved. These objectives would then form the basis of the experimental design of the study, and the methodology to be adopted for its execution. These aspects have been detailed below, in the sequence explained above.